Common WU polyomavirus infection in a Beijing population indicated by surveillance for serum IgG antibody against capsid protein VP2

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Original article

Background: **WU polyomavirus (WU virus) was identified as a novel polyomavirus in 2007 from specimens of pediatric patients with acute respiratory infection (ARI). A lack of permissive cell lines has limited investigations into WU virus pathogenesis and prevalence.**

Methods: **The encoding region of the capsid protein VP2 gene was amplified from a WU virus DNA-positive clinical specimen and expressed as a recombinant His-tagged protein in *Escherichia coli* BL21 (DE3). The expressed VP2 was identified by expected molecular weight and immunoreactivity with anti-His monoclonal antibody in Western blotting assay. Serum samples collected from 455 individuals of all ages in Beijing without symptoms of ARI were tested for IgG antibodies against the affinity-purified recombinant VP2 protein by Western blotting to investigate the prevalence of natural WU virus infection. In addition, serum samples from four ARI pediatric patients, whose nasopharyngeal aspirates were positive for WU virus DNA and negative for all other respiratory-related viruses, were tested for IgM antibody against the recombinant VP2.**

Results: **Of the 455 serum samples, 238 reacted with the recombinant VP2, yielding an overall positive rate of 52.3% for IgG against VP2 of WU virus. The positive rate was the highest in serum samples from infants and children between 1 to 4 years of age. One of four ARI pediatric patients was positive for IgM against WU virus VP2, implicating WU virus as the causative disease agent.**

Conclusions: **The high prevalence of IgG against WU polyomavirus in Beijing-based study population indicates that WU virus infection is common in Beijing. WU virus may be responsible for some pediatric ARI cases, and primary infection of this virus may occur mostly in childhood.**

World J Pediatr 2013;9(1):48-52

Key words: **antibody;**
infection;
virus;
WU polyomavirus

Introduction

A novel human polyomavirus was identified in the USA in 2007 by using high-throughput sequencing, and designated as the WU polyomavirus (WU virus).[1] The human polyomaviruses include BK virus (BKV), JC virus (JCV), KI polyomavirus (KI virus), WU virus, Merkel Cell polyomavirus (MCPyV), human polyomavirus 6 and 7 (HPyV 6,7), and trichodysplasia spinulosa-associated polyomavirus (TSV).[1-7] The WU virus is a small, non-enveloped double-strand (ds) DNA virus with a covalent closed circular genome of 5229 bp. The viral capsid of WU virus comprises three structural proteins: VP1 [369 amino acids (aa)], VP2 (415 aa; 43.6 kDa), and VP3 (272 aa).

The WU virus was initially detected in a nasopharyngeal aspirate (NPA) from a 3-year-old Australian child diagnosed with pneumonia. Subsequent studies using polymerase chain reaction (PCR) have detected WU virus DNA in NPA samples[8-10] of patients with acute respiratory infection (ARI) and in other non-NPA samples, such as tissues and stools[11-13] of patients without respiratory symptoms. Therefore, the pathogenic and epidemiologic profiles of the WU virus remain unknown.

In our laboratory, we detected WU virus DNA in 5.6% (of 674) NPAs collected from pediatric patients with ARI and in 1.5% (of 202) NPAs from children without ARI, suggesting that WU virus might be a causative pathogen of ARI in children in Beijing.[14] However, serological evidence should be demonstrated to verify this hypothesis. A lack of cell lines sensitive

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doi: 10.1007/s12519-013-0397-1

to WU virus replication and insufficient amounts of WU virus that may be used as an antigen in a serological study have limited further investigations. However, a WU virus recombinant protein may be useful as an alternative antigen in serological studies.

In the present study, recombinant VP2 was expressed in *Escherichia coli* BL21 (DE3) and it was used as an antigen to detect antibodies against WU virus in a representative healthy population of Beijing. In addition, the recombinant VP2 was used to detect specific antibody response in serum samples from pediatric patients with ARI, whose NPA specimens were WU virus DNA positive.

Methods

Serum specimens

Serum specimens were collected from 455 individuals, including 179 adults (≥18-year-old) who presented for a regular health check-up at a clinic in Beijing, China in August 2005 and 276 pediatric patients (<18-year-old) with symptoms other than ARI who were admitted to the Children's Hospital Affiliated to the Capital Institute of Pediatrics (Beijing, China) from August 2009 to June 2010. In addition, serum specimens were collected from four pediatric patients with ARI whose NPAs were confirmed positive for WU virus DNA and negative for all other respiratory viruses. Direct fluorescence assays (DFA; D3® UltraTM DFA Respiratory Virus Screening & ID Kit; Diagnostic Hybrids, Athens, OH, USA) were used to screen for respiratory syncytial virus (RSV), influenza virus types A and B (Inf A and B), parainfluenza virus types 1, 2 and 3 (PIV 1-3), and adenoviruses (AdVs).[14] Reverse transcription-polymerase chain reaction (RT-PCR) or standard PCR were used to screen for parainfluenza virus type 4 (PIV 4), human metapneumovirus (HMPV), human rhinovirus (HRV), human bocavirus (HBoV), and human coronaviruses (HCoV) NL63, OC43 and 299E.[15-19]

All sera were stored at -20°C before testing. This study was approved by the Medical Ethics Committee of the Capital Institute of Pediatrics.

Primers used to amplify the VP2 encoding gene

Primers were designed according to the published sequences of the VP2 encoding genes from WU virus strains B0 (GenBank accession no. EF444549)[1] and BJF5276 from Beijing (GenBank accession no. EU693903.1)[14]: WU-VP2S, 5′-TATTGGTGCTACCGTCTCG-3′; WU-VP2A, 5′-CTTCAGCAGTTTTAAGTGGG-3′; WU-VP2F, 5′-CAGGATCCATGGGCATATTGCTTGCT-3′ with a *BamH* I site; and WU-VP2R, 5′-CGGGCTCGAGT TAAACTCTGTTTCTTCT-3′ with an Xhol I site.

PCR amplification of the VP2 encoding region of WU virus

The gene fragment of the VP2 encoding region of WU virus was amplified by nested PCR from a specimen (BJF5276) confirmed positive for WU virus DNA in a previous study.[14] Primers WU-VP2S and WU-VP2A were used in the first PCR run, and primers WU-VP2F and WU-VP2R were used in the second PCR run. The resultant amplicon of the expected size (1264 bp) was purified using an Easypure Quick Gel Extraction kit (EG 101; Transgen Biotech, Beijing, China).

Generation of recombinant plasmid WU VP2-pET30a and expression of WU virus VP2

The purified VP2 encoding gene and the plasmid pET30a (+) with 6×His tag (No. 69909-3: Merck-Novagen, Darmstadt, Germany) were digested by *BamH* I and *Xho* I (Promega, Madison, WI, USA) and ligated to generate the recombinant plasmid VP2-pET30a. The recombinant plasmid was verified by sequencing and transformed into *E. coli* BL21 (DE3) competent cells. The expression of recombinant VP2 protein with 6×His tag (49 kDa) was induced by exposure to 1 mmol/L isopropyl-β-D-thiogalactopyranoside (IPTG) and verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie brilliant blue staining for visualization. The expression of the recombinant VP2 was determined by Western blotting using mouse anti-His monoclonal primary antibody (1:4000 dilution, No. 34670: Qiagen, Heidelberg, Germany) and horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG secondary antibody (1:4000; Jackson ImmunoResearch, West Grove, PA, USA).

The expressed recombinant VP2 protein was affinity-semi-purified with nickel-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen) following the manufacturer's protocol. *E. coli* BL21 (DE3) transformed with PET30a vector alone was used as a negative control (mock).

Western blotting assay for detection of human serum antibodies against recombinant VP2 of WU virus

The affinity-purified recombinant VP2 protein of WU virus was separated by SDS-PAGE using a 10% polyacrylamide gel and electro-transferred onto a nitrocellulose membrane, which was then sliced into strips and used as an antigen in Western blotting assays. All the 455 serum samples were diluted to 1:200 for specific IgG testing, and the four serum samples from pediatric ARI patients were diluted to 1:10 for specific IgM testing. After incubating the recombinant VP2 blotted strips with the diluted samples, the strips were reacted with either HRP-conjugated goat anti-human IgG

(1:4000; Jackson ImmunoResearch) or goat anti-human IgM antibodies (1:5000; Jackson ImmunoResearch). Antibody binding with the recombinant VP2 was visualized as a 49 kDa molecular weight band.

Results

Expression of WU virus VP2 in *E. coli* BL21

The recombinant VP2 protein (49 kDa) expressed in *E. coli* BL21 (DE3) by the recombinant plasmid WU VP2-pET30a was collected after IPTG induction for 2, 4, 6, 8 and 10 hours, respectively. The recombinant protein samples were evaluated by SDS-PAGE and Coomassie brilliant blue staining (Fig. 1A, lanes 3-7). The quantity of the recombinant VP2 protein increased with induction time, and peaked after 10-hour induction. The mock control cells expressed no protein of 49 kDa size (Fig. 1A, lane 1). The 49 kDa recombinant protein was evaluated by Western blotting assay using anti-His monoclonal antibody after IPTG induction for 2, 4, 6, 8 and 10 hours, respectively (Fig. 1B, lanes 3-7). No immunoreactivity was seen in the mock control cells (Fig.1B, lane 1), confirming that the recombinant VP2 of WU virus was expressed in the transformed *E. coli* BL21 cells.

Western blotting of human serum IgG or IgM antibodies against the recombinant VP2 of WU virus

The recombinant VP2 protein purified by Ni-NTA was used as an antigen to detect serum IgG or IgM antibodies against WU virus by Western blotting assay. Using the anti-His monoclonal IgG (Fig. 2A) as positive control, the serum specimens showed immunoreactive bands of 49 kDa in size. Samples with the 49 kDa immunoreactive band were considered positive for specific IgG or IgM against WU virus VP2 (Fig. 2B).

The serum positive rates for IgG against WU virus VP2 are shown in Table 1. Of 455 specimens tested, 52.3% (238/455) were positive for IgG against WU virus VP2, including 48.6% (87/179) of adults and 54.7% (151/276) of children. For infants younger than six months, the positive rate was 51.2%. The positive rate increased with age in children (Table).

All four of the serum specimens collected from hospitalized children with ARI, whose NPAs were positive for WU virus DNA and negative for other respiratory viruses, were positive for IgG against WU virus VP2. However, only one specimen was positive for IgM against WU virus VP2.

Discussion

The lack of cell lines sensitive to WU virus replication and insufficient WU virus amounts produced under laboratory conditions have impeded serological studies. To solve this problem, we generated a recombinant VP2 of WU virus using an *E. coli* BL21 expression system and applied this protein as an antigen to successfully detect specific antibody against WU virus in human sera. Our findings have provided serological evidence of natural WU infection in adults and children from Beijing, China.

Table. Age distribution of IgG seropositivity against WU virus VP2

Age	IgG seropositivity
	Percentage of positive samples, % (positive/tested)
<6 mon	51.2 (21/41)
6 mon	30.6 (11/36)
1 y-	48.5 (16/33)
2 y-	57.6 (19/33)
3 y-	75.0 (24/32)
4 y-	83.3 (25/30)
5-10 y	58.3 (21/36)
10-17 y	40.0 (14/35)
18-28 y	46.7 (14/30)
29-39 y	57.9 (22/38)
40-49 y	36.1 (13/36)
50-59 y	47.2 (17/36)
60-81 y	53.8 (21/39)
Total	52.3 (238/455)

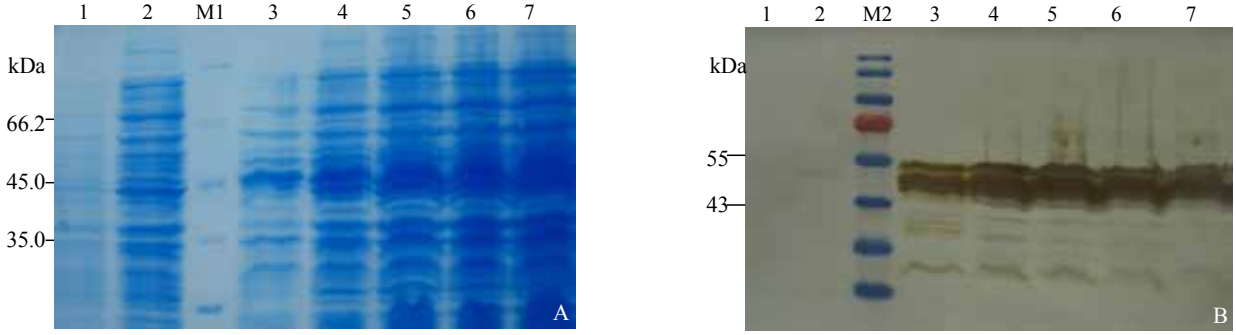

Fig. 1. Expression of WU virus VP2 in *E. coli* BL21 (DE3). **A**: SDS-PAGE with Coomassie brilliant blue staining; **B**: Western blot assay with anti-His antibody. Lane 1: mock cells transformed with pET30a vector; Lanes 2-7: cells transformed with WU VP2-pET30a vector and detected after IPTG induction at 0, 2, 4, 6, 8 and 10 hours, respectively.

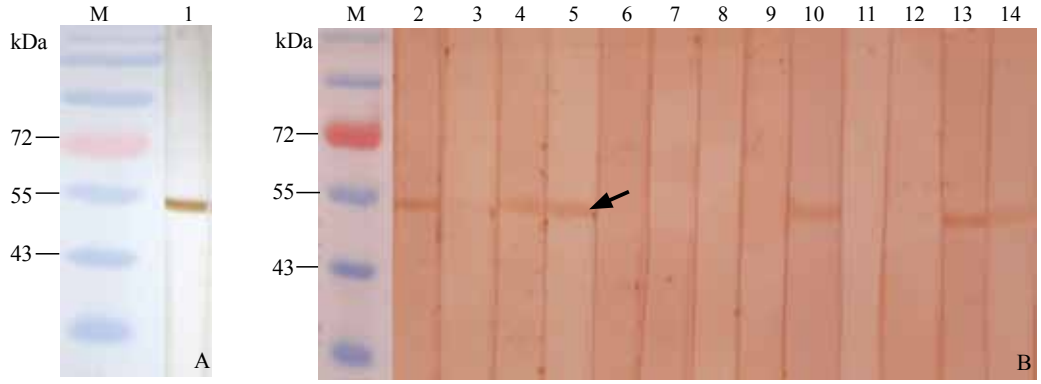

Fig. 2. Purified recombinant VP2 of WU virus reacted with anti-His monoclonal IgG as a positive control (**A**, lane 1) and (**B**, 13 serum specimens for the detection of specific IgG against WU virus VP2 (lanes 2-14), and 6 serum specimens with specific bands in lanes 2, 4, 5, 10, 13 and 14 indicated by arrow were positive for IgG against WU VP2.

To date, no specific antibody against WU virus has been available commercially. Since the pET30a (+) vector has a 6×His tag, the expression of recombinant WU virus VP2 was detected by an anti-His monoclonal antibody. Western blotting analysis indicated that the affinity-purified recombinant VP2 protein bound specifically to mouse anti-His monoclonal antibody. No immunoreactivity was detected in the mock controls. These data indicated that the recombinant protein with 6×His tag was specifically effective as antigen for detecting the antibodies against recombinant WU virus VP2 protein.

Considering the low amino acid sequence identities among the VP2s of WU virus and BKV/JCV (17%/16%), we speculate that very little cross-reactivity exists between the various VP2s. Kantola et al[20] demonstrated that there was no cross-reactivity of VP2 protein between WU virus and BKV/JCV in a serology-based study. No study has demonstrated the cross-reactivity of VP2s from WU virus and KI virus, probably because of the lack of a specific antibody. It has been reported, however, that the VP1s of WU virus and KI virus share 65% identity in amino acid sequences, yet previous competition assay and Western blotting revealed minimal, if any, detectable cross-reactivity between the two viruses.[21,22] The VP2s of WU virus and KI virus share 71% identity in amino acid sequences, and more work should be done to determine whether cross-reactivity exists among this structural protein from the two viruses.

To gain insights into the prevalence of WU virus infection in Beijing, serum antibodies against WU virus VP2 were examined by Western blot assays using recombinant VP2 as antigen. The high overall serum positive rate for IgG against WU virus VP2 (52.3%) suggests that infection by WU virus is common among the Beijing population. In the present study, the sero-positive rate of IgG antibodies against VP2 was 54.7% in the age group <18 years, and about 48.6% in the population ≥18 years. The positive rate in the age group <6 months was 51.2%, which was similar to that of adults (≥18 years). It was suggested that IgG antibodies against WU virus VP2 in this age group may have been maternally transmitted. The sero-positive rate for WU virus VP2 decreased to a nadir in the next age group (6 months to <1 year), then steadily increased to a peak level (83.3%), and subsequently decreased and plateaued at less than 50%. These data implied that infection of WU virus was most prevalent in infants and children between 1 and 4 years of age and suggested that primary infection by WU virus occurred mainly in early childhood. Furthermore, this result was consistent with that from a previous study in our laboratory, in which serology data suggested that WU virus is a pathogen of ARI in children in Beijing.[14]

Serum sample that was positive for IgM against WU virus VP2 was obtained from a 1-year-old boy. He was diagnosed with bronchial pneumonia on the 7th day after symptom onset, and his NPA was positive for WU virus DNA. Furthermore, the NPA was negative for common and other respiratory viruses, including RSV, InfA and B, PIV 1-3, ADVs, PIV4, HMPV, HRV, HBoV, and HCoV NL63, OC43, and 229E. These data supported the assumption that the WU virus, at least partially, contributed to the patient's respiratory infection.

There are some limitations in this study that should be considered when interpreting our results. First, we were unable to investigate cross-reactivity among antibodies against VP2 proteins from WU virus, BKV, JCV and KI virus because of the lack of specific antibodies against these viruses. Second, 276 serum specimens were collected from pediatric patients with symptoms other than ARI and not from healthy children because serum samples from healthy children were not available for testing because of ethical implications, which may complicate the results. Nonetheless, the data from this study represent a preliminary sero-prevalence

profile of naturally acquired WU virus infections. In addition, this study provides the first serology data on WU virus from a non-Western country.

In conclusion, this survey of serum antibodies against WU virus in a Beijing population using a recombinant capsid protein VP2 in Western blotting assay revealed that WU virus infection appears to be common in Beijing. In addition, WU virus is implicated as the pathogenic agent of ARI in some patients. Finally, primary infection of WU virus is likely to occur in childhood.

Acknowledgements

The authors would like to express their gratitude to the clinicians and nurses of the Affiliated Children's Hospital to the Capital Institute of Pediatrics, Beijing, China, for collecting clinical samples and patient information.

Funding: This work was supported by grants from the National Natural Science Foundation of China (No. 30872153) and the Beijing Municipal Health Bureau "Advanced Personnel Training Program" (No. 2011-3-068).
Ethical approval: This study was approved by the Medicine Ethics Committee of the Capital Institute of Pediatrics.
Competing interest: None declared.
Contributors: Zhang NN and Zhao LQ contributed equally to this paper. Zhang NN carried out most of the experiments in this study and wrote the original manuscript. Zhao LQ participated in the design of the experimental approach, constructed the recombinant plasmid with the full-length VP2 gene, and reviewed the manuscript. Qian Y was the principal investigator of this research project and reviewed the manuscript. All other authors conducted focused experiments or data analysis.

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Received November 15, 2011
Accepted after revision May 10, 2012